Product Datasheet

INSIG-1 Antibody - BSA Free
NB110-55244

Unit Size: 0.1 ml

Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.

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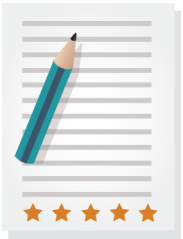
Updated 10/23/2024 v.20.1



NB110-55244

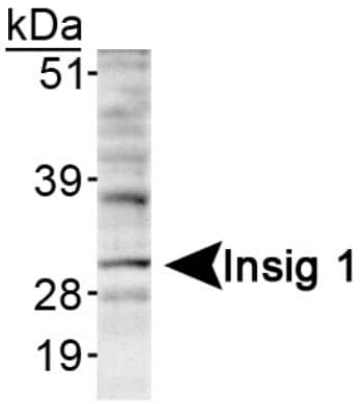
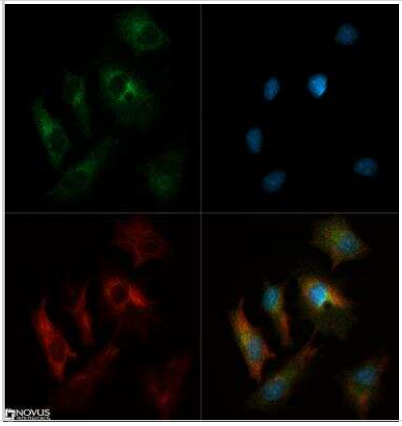
INSIG-1 Antibody - BSA Free

Product Information	
Unit Size	0.1 ml
Concentration	1.02 mg/ml
Storage	Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.1% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS and 30% Glycerol
Target Molecular Weight	30 kDa

Product Description	
Host	Rabbit
Gene ID	3638
Gene Symbol	INSIG1
Species	Human, Rat
Specificity/Sensitivity	This is specific for Insig1 but not Insig2.
Immunogen	A synthetic peptide representing an internal region of the human INSIG-1 protein (between residues 1-100) according to Swiss-Prot# O15503.

Product Application Details	
Applications	Western Blot, Immunocytochemistry/ Immunofluorescence
Recommended Dilutions	Western Blot 2 ug/ml, Immunocytochemistry/ Immunofluorescence 1:100-1:1000
Application Notes	This INSIG-1 antibody is useful for Immunocytochemistry/Immunofluorescence and Western Blot, where a band is seen at ~30 kDa representing the Insig1 protein. In ICC/IF positive staining was localized to the ER membrane in HepG2 cells. The observed molecular weight of the protein may vary from the listed predicted molecular weight due to post translational modifications, post translation cleavages, relative charges, and other experimental factors.

Images

Western Blot: INSIG-1 Antibody [NB110-55244] - Detection of Insig 1 in HepG2 lyaste using NB110-55244.	
Immunocytochemistry/Immunofluorescence: INSIG-1 Antibody [NB110-55244] - INSIG-1 antibody was tested in HepG2 cells with Dylight 488 (green). Nuclei and alpha-tubulin were counterstained with DAPI (blue) and Dylight 550 (red).	

Publications

Egawa N, Izumi Y, Suzuki H et al. TDP-43 regulates cholesterol biosynthesis by inhibiting sterol regulatory element-binding protein 2 Scientific Reports 2022-05-14 [PMID: 35568729] (Western Blot, Block/Neutralize)

Nakanishi T, Tanaka R, Tonai S Et al. LH induces de novo cholesterol biosynthesis via SREBP activation in granulosa cells during ovulation in female mice Endocrinology 2021-08-25 [PMID: 34431998]

Beehler K MiR-1908 Is a Cholesterol Responsive MicroRNA Implicated In Cholesterol Regulation Thesis 2020-01-01 (WB, Human)

Segatto M, Di Giovanni AL, Marino M, Pallottini V. Analysis of the protein network of cholesterol homeostasis in different brain regions: an age and sex dependent perspective J Cell Physiol 2012-12-31 [PMID: 23280554] (WB, Rat)

Trapani L, Segatto M, Simeoni V et al. Short- and long-term regulation of 3-hydroxy 3-methylglutaryl coenzyme A reductase by a 4-methylcoumarin Biochimie 2011-07-01 [PMID: 21530605] (WB, Human)

De Marinis E, Martini C, Trentalance A et al. Sex differences in hepatic regulation of cholesterol homeostasis. J Endocrinol;198(3):635-43. 2008-09-01 [PMID: 18603607]

Segatto M, Trapani L, Lecis C, Pallottini V. Regulation of cholesterol biosynthetic pathway in different regions of the rat central nervous system. Acta Physiol (Oxf). 2012-05-16 [PMID: 22591135] (WB, Rat)

Procedures

Western Blot protocol for INSIG-1 Antibody (NB110-55244)
INSIG-1 Antibody:
Western Blot Protocol

1. Perform SDS-PAGE (4-12% MOPS) on samples to be analyzed, loading 30 ug of total protein per lane.
2. Transfer proteins to Nitrocellulose according to the instructions provided by the manufacturer of the transfer apparatus.
3. Rinse membrane with dH2O and then stain the blot using Ponceau S for 1-2 minutes to access the transfer of proteins onto the nitrocellulose membrane. Rinse the blot in water to remove excess stain and mark the lane locations and locations of molecular weight markers using a pencil.
4. Rinse the blot in TBS for approximately 5 minutes.
5. Block the membrane using 5% non-fat dry milk + 1% BSA in TBS, 1 hour at room temperature.
6. Rinse the membrane in dH2O and then wash the membrane in wash buffer [TBS + 0.1% Tween] 3 times for 10 minutes each.
7. Dilute the rabbit anti-Insig1 primary antibody (NB 110-55244) in blocking buffer and incubate 2 hours at room temperature.
8. Rinse the membrane in dH2O and then wash the membrane in wash buffer [TBS + 0.1% Tween] 3 times for 10 minutes each.
9. Apply the diluted rabbit-IgG HRP-conjugated secondary antibody in blocking buffer (as per manufacturers instructions) and incubate 1 hour at room temperature.
10. Wash the blot in wash buffer [TBS + 0.1% Tween] 3 times for 10 minutes each (this step can be repeated as required to reduce background).
11. Apply the detection reagent of choice in accordance with the manufacturers instructions (Pierce, ECL).

Note: Tween-20 can be added to the blocking or antibody diultion buffer at a final concentration of 0.05-0.2%, provided it does not interfere with antibody-antigen binding.

Immunocytochemistry/Immunofluorescence protocol for INSIG-1 Antibody (NB110-55244)
INSIG-1 Antibody:
Immunocytochemistry Protocol

Culture cells to appropriate density in 35 mm culture dishes or 6-well plates.

1. Remove culture medium and add 10% formalin to the dish. Fix at room temperature for 30 minutes.
2. Remove the formalin and add ice cold methanol. Incubate for 5-10 minutes.
3. Remove methanol and add washing solution (i.e. PBS). Be sure to not let the specimen dry out. Wash three times for 10 minutes.
4. To block nonspecific antibody binding incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
5. Add primary antibody at appropriate dilution and incubate at room temperature from 2 hours to overnight at room temperature.
6. Remove primary antibody and replace with washing solution. Wash three times for 10 minutes.
7. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
8. Remove antibody and replace with wash solution, then wash for 10 minutes. Add Hoechst 33258 to wash solution at 1:25,0000 and incubate for 10 minutes. Wash a third time for 10 minutes.
9. Cells can be viewed directly after washing. The plates can also be stored in PBS containing Azide covered in Parafilm (TM). Cells can also be cover-slipped using Fluoromount, with appropriate sealing.

*The above information is only intended as a guide. The researcher should determine what protocol best meets their needs. Please follow safe laboratory procedures.

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Products Related to NB110-55244

NBL1-12003	INSIG-1 Overexpression Lysate
HAF008	Goat anti-Rabbit IgG Secondary Antibody [HRP]
NB7160	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]
NBP2-24891	Rabbit IgG Isotype Control

Limitations

This product is for research use only and is not approved for use in humans or in clinical diagnosis. Primary Antibodies are guaranteed for 1 year from date of receipt.

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